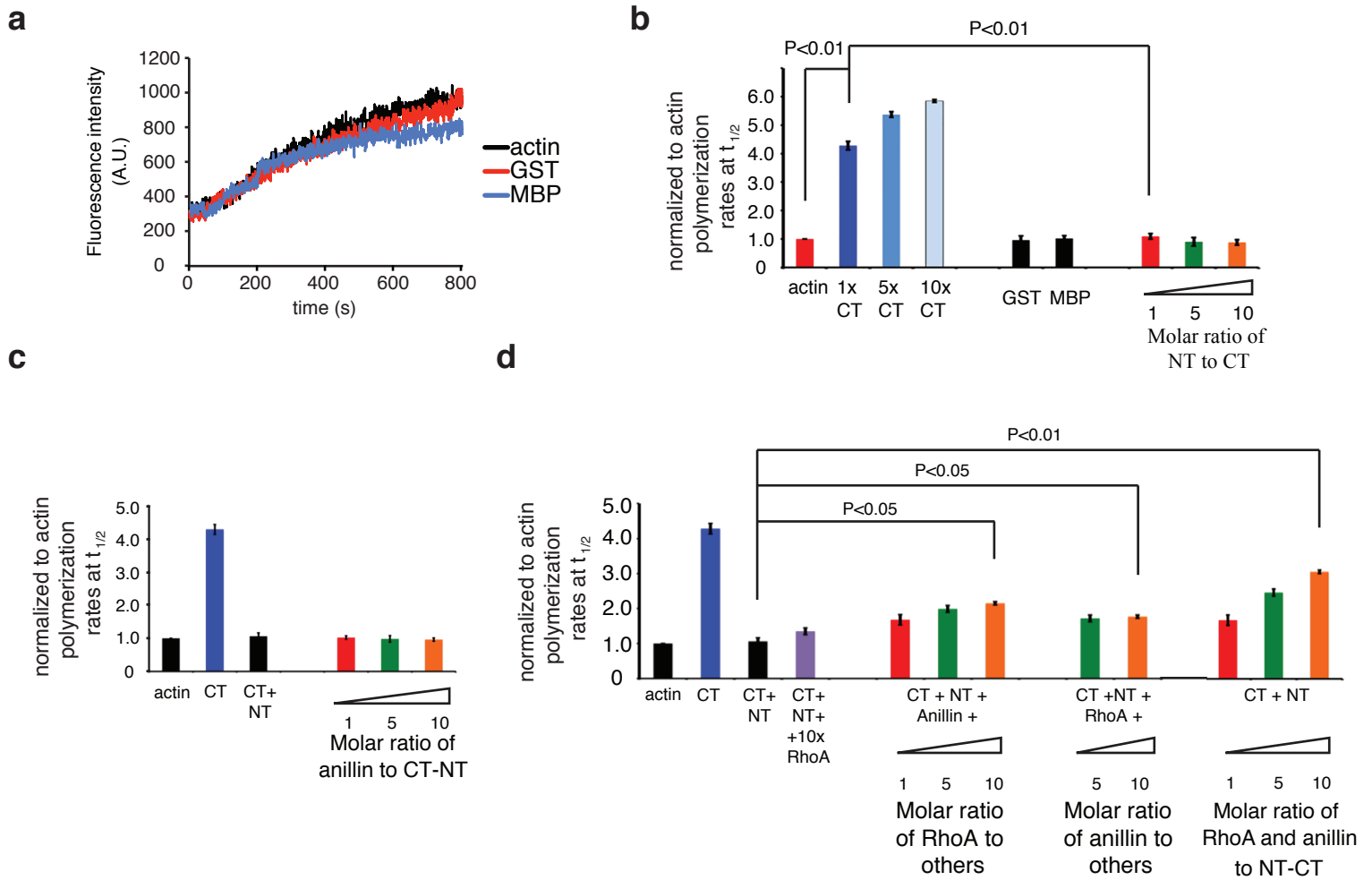




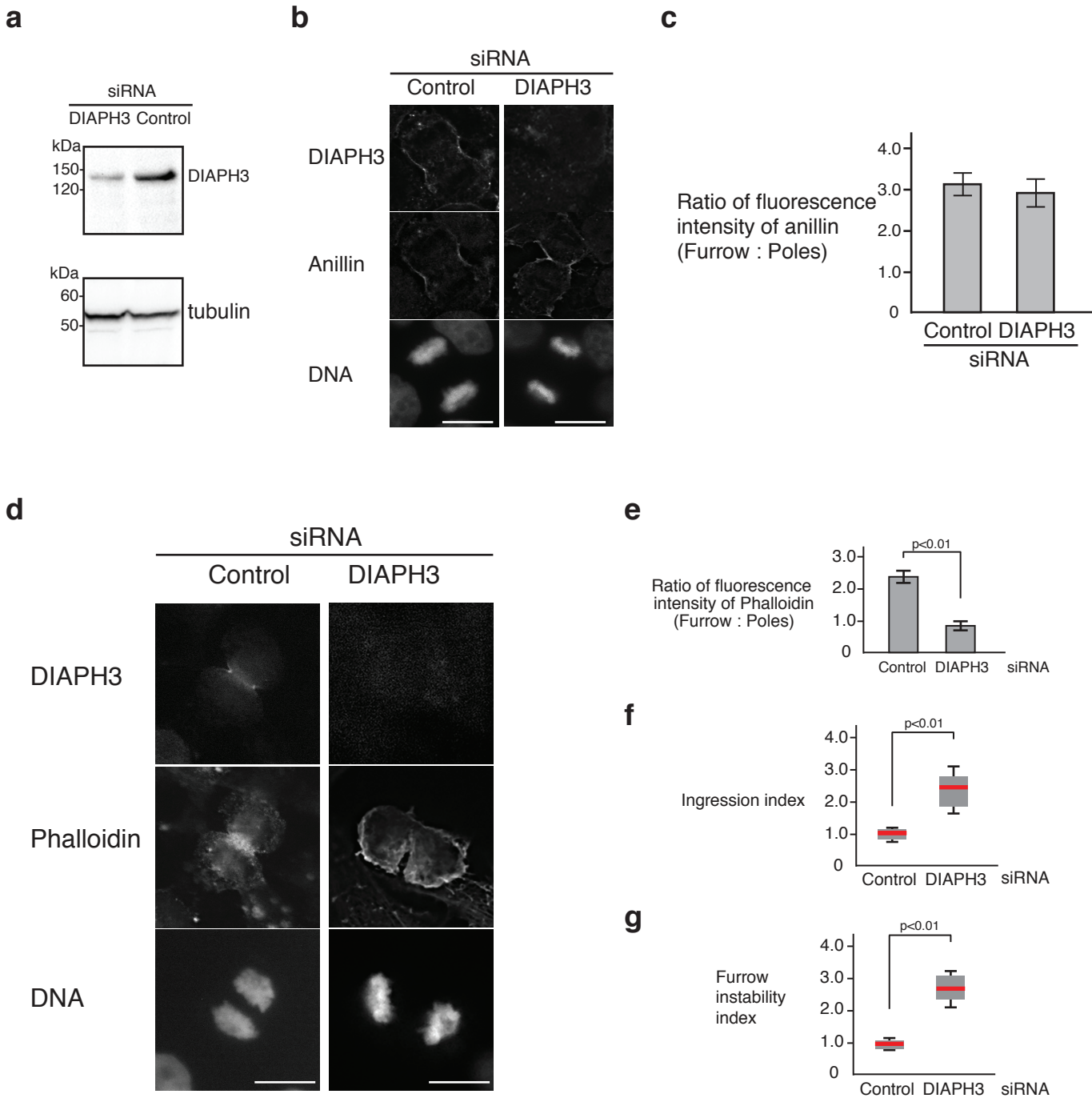
Supplementary Figure 2. Comparative pyrene actin polymerization assays.

(a) Pyrene-labeled actin polymerization rates alone and in the presence of purified recombinant MBP and GST.

(b) Effects of different concentrations of MBP-DIAPH3 CT in the presence and absence of GST-DIAPH 3 NT on the rate of actin polymerization. Normalized actin polymerization rates at $t_{1/2}$ maximum relative to $t_{1/2}$ maximum of actin alone. $n=3$.

(c) Effects of increasing concentration of 6xHis anillin 1-151 on actin polymerization in the presence of pre-incubated MBP-DIAPH3 CT and GST-DIAPH 3 NT. $n=3$.

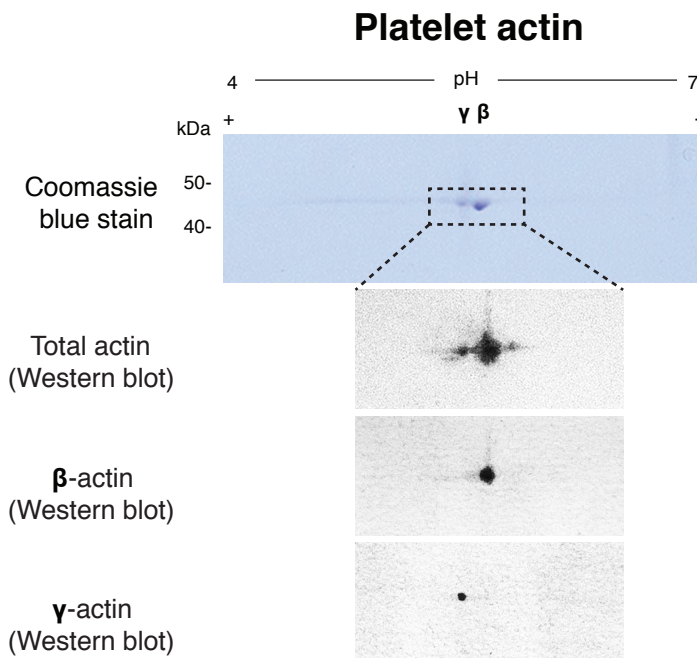
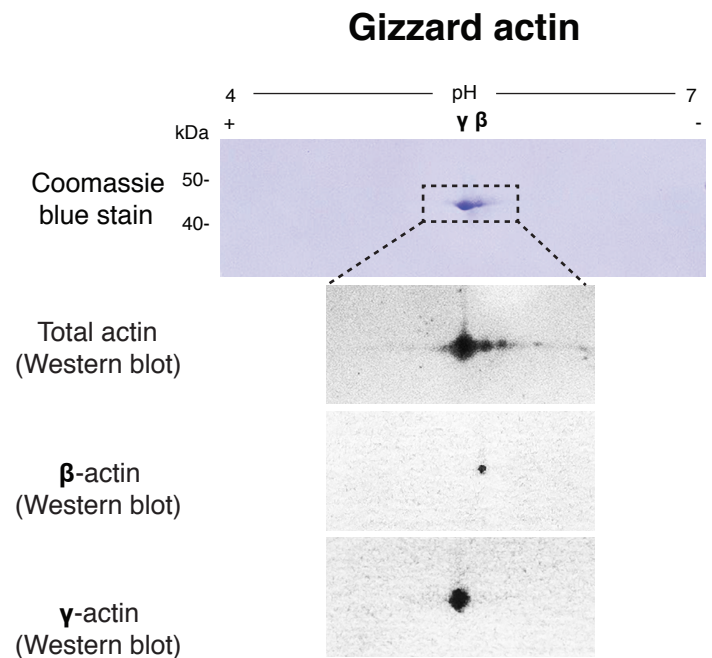
Error bars are $\pm$ s.e.m. **(d)** Effects on actin polymerization in the presence of a constant concentration of pre-incubated MBP-DIAPH3 CT and GST-DIAPH 3 NT but varying concentrations of GST RhoA, 6xHis anillin (1-151) or both. $n=3$. Error bars are $\pm$ s.e.m. Student's t -test was used for statistical analysis.



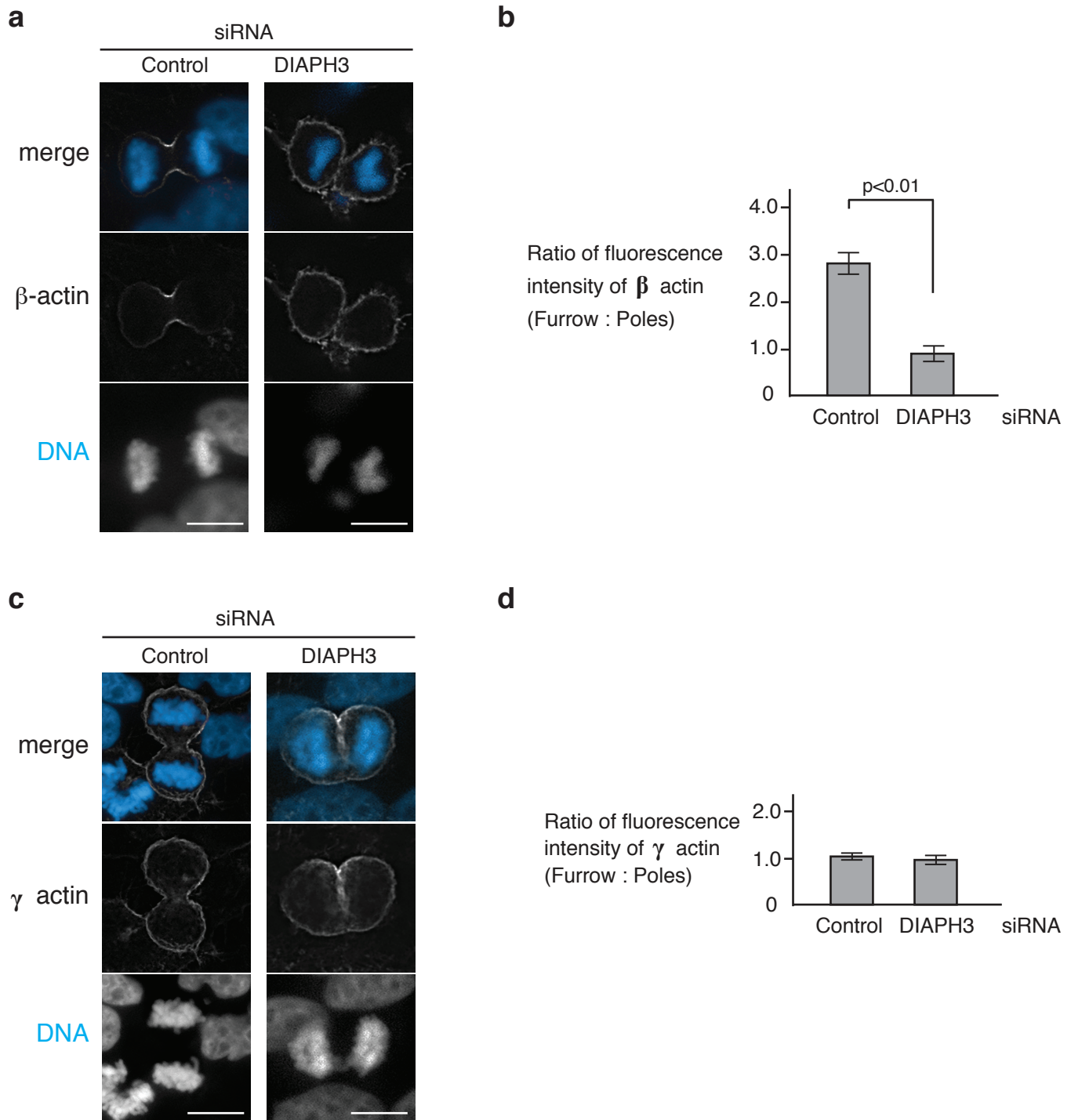
Supplementary Figure 3. Depletion of DIAPH3 disrupts cytokinetic actin and furrow organization. **(a)** Western blot showing the extent of DIAPH3 depletion upon siRNA treatment after 48h. **(b)** Control and DIAPH3 siRNA treated HeLa cell fixed and stained with DIAPH3, anillin antibodies and DAPI. **(c)** Quantification of the relative distribution of anillin, furrow to pole, in cells treated in b. At least 100 cells were counted in three different experiments. Error bars are $\pm$ s.e.m. **(d)** Cells treated with control and DIAPH siRNA stained with phalloidin and DAPI to determine the relative distribution of actin filaments in cytokinetic cells. **(e)** Quantification of actin distribution in cells described in e. At least 100 cells were counted in three different experiments. Error bars are $\pm$ s.e.m. **(f)** Furrow ingression index of cells treated with control and DIAPH3 siRNA. At least 100 cells were counted in three different experiments. Error bars are $\pm$ s.e.m. **(g)** Furrow stability index of cells treated with control and DIAPH3 siRNA. Error bars are $\pm$ s.e.m. Student's *t*-test was used for statistical analysis. All scale bars are 10 μ m.

a

α -actin	human	MCDEDETTALVCDNGSGLVK
	mouse	MCDEDETTALVCDNGSGLVK
	chicken	MCDEDETTALVCDNGSGLVK
	Xenopus	MCDEDETTALVCDNGSGLVK
cytoplasmic β -actin	human	MDD DIAALVVDNGSGMCK
	mouse	MDD DIAALVVDNGSGMCK
	chicken	MDD DIAALVVDNGSGMCK
	Xenopus	MDD DIAALVVDNGSGMCK
cytoplasmic γ -actin	human	MEEE IAALVIDNGSGMCK
	mouse	MEEE IAALVIDNGSGMCK
	chicken	MEEE IAALVIDNGSGMCK
	Xenopus	MEEE IAALVIDNGSGMCK

b**c**

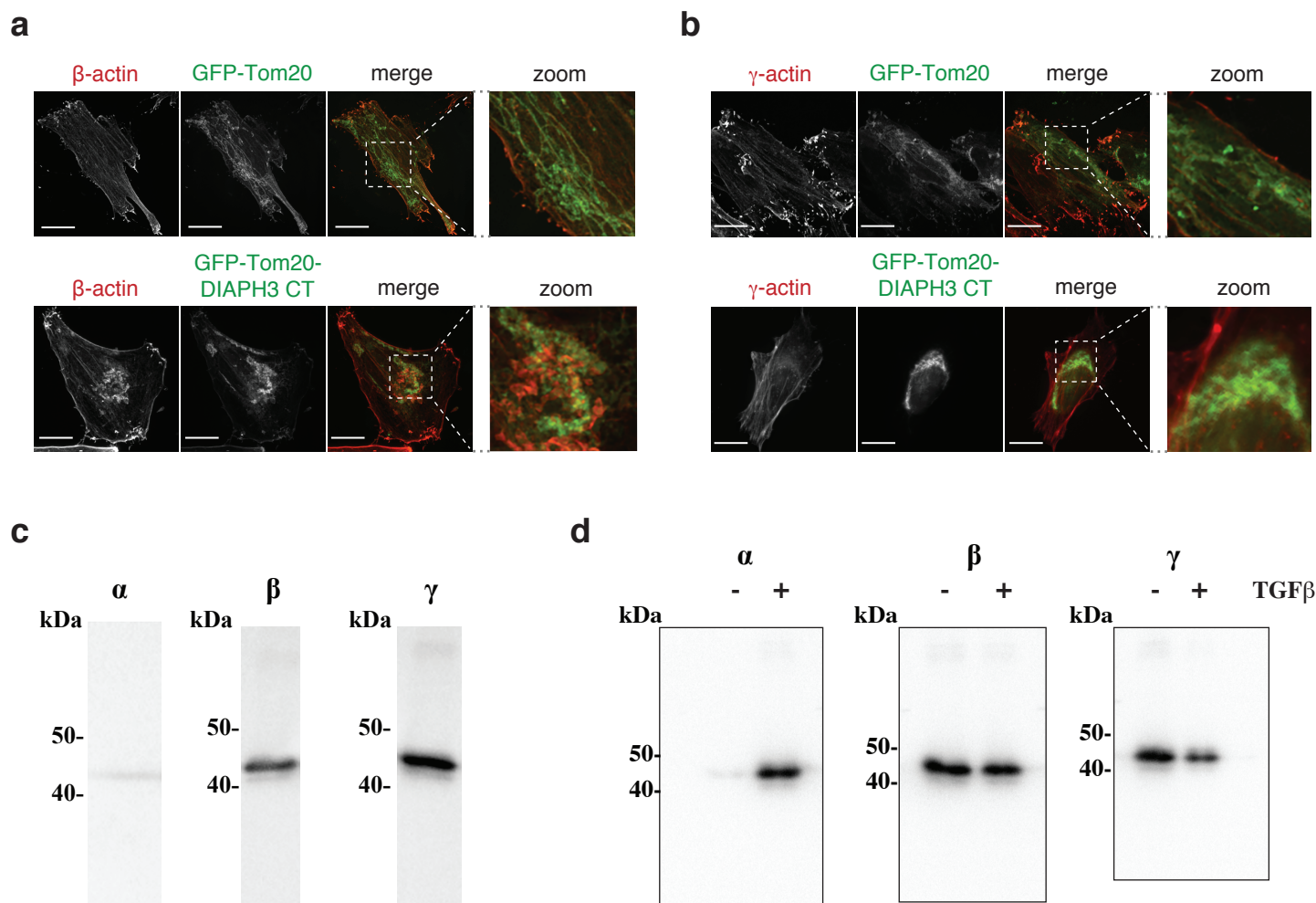
Supplementary Figure 4. Comparison of actin isoforms. **(a)** Cross species amino acid sequence alignment of the N-terminal 20 amino acids of different actin isoforms. Blue indicates residues conserved across isoforms and species. Pink and orange boxes highlight the divergent acidic amino acids at the N-terminus of the different actin isoforms. Gray boxes indicate residues conserved between β and γ , but not with α . **(b)** 2-D (isoelectric focusing, pH range 4-7, followed by SDS-PAGE) analysis of actin from human platelets. Top panel Coomassie stained gel, lower panels western blots probed with antibodies recognizing all actin isoforms, β -actin isoform specific and γ -actin isoform specific. **(c)** 2-D (isoelectric focusing, pH range 4-7, followed by SDS-PAGE) analysis of actin from chicken gizzards. Top panel Coomassie stained gel, lower panels western blots probed with antibodies recognizing all actin isoforms, β -actin isoform specific and γ -actin isoform specific.



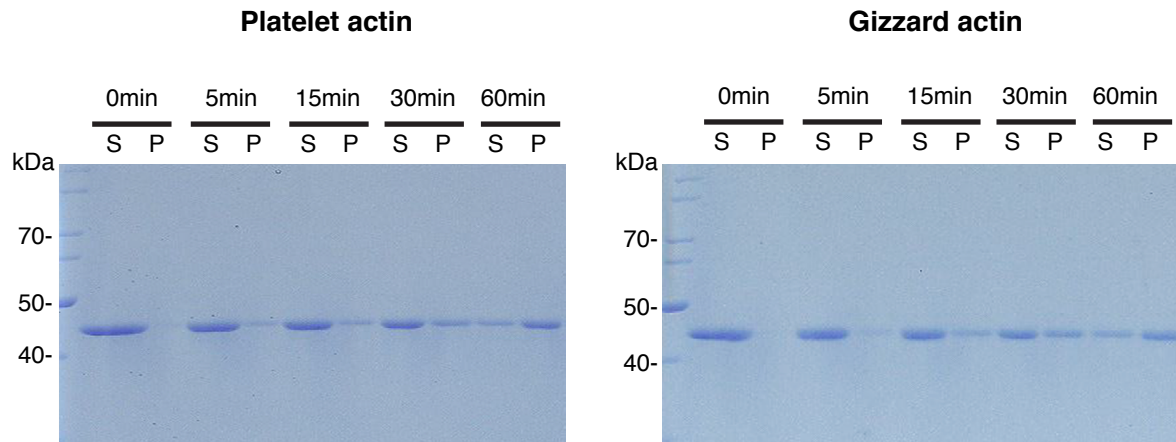
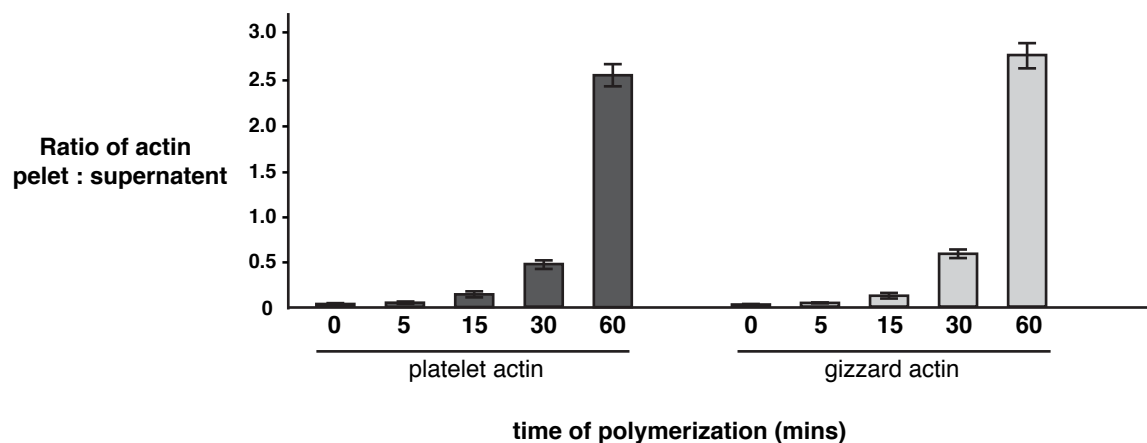
Supplementary Figure 5. Depletion of DIAPH3 disrupts cytokinetic β -actin organization.

(a) HeLa cells treated with control and DIAPH3 siRNA, fixed and stained with DIAPH3 and β -actin antibodies. **(b)** Quantification of the distribution β -actin in the cell described in a. At least 100 cells were counted in three different experiments. Error bars are $\pm$ s.e.m. Student's *t*-test was used.

(c) HeLa cells treated with control and DIAPH3 siRNA, fixed and stained with DIAPH3 and γ -actin antibodies. **(d)** Quantification of the distribution γ -actin in the cell described in c. At least 100 cells were counted in three different experiments. Error bars are $\pm$ s.e.m. All scale bars are 10 μ m.



Supplementary Figure 6. Ectopic targeting of DIAPH3 to the surface of mitochondria stimulates the localized production of β -actin but not γ -actin. The C-terminal region of DIAPH3 that contains the FH1 domain and the FH2 actin nucleation domain was fused to GFP and the mitochondrial-targeting element of TOM20 then transfected into HeLa cells. Cells were fixed 24 hours later and stained with antibodies that specifically recognize **(a)** β - and **(b)** γ -actin. Scale bars are 10 μ m. **(c)** Immunoblot of HeLa cell lysates probed with α -, β - and γ -actin specific antibodies. **(d)** Immunoblot of MRC-5 cell lysates, generated from cells grown in the absence or presence of 2ng/ml TGF β for 96 hours to induce the expression of α -actin, probed with α -, β - and γ -actin specific antibodies.

a**b**

Supplementary Figure 7. Comparative polymerization activity of actin isolated from human platelets and chicken gizzards. Actin from the different sources was incubated in polymerization buffer and at different time points centrifuged at 65,000rpm for 20min in a TLA 120.2 rotor applied in Beckman TL-100 Ultracentrifuge (Beckman Coulter, Inc.). **(a)** The amount of actin in the supernatant (S), unpolymerized actin was compared to the amount of actin in the pellet (P), polymerized actin by SDS-PAGE and coomassie blue staining. **(b)** The comparative amount of actin in the pellet and supernatant fractions was determined by densitometry measurements of the Coomassie blue stained SDS PAGE gels in a. n=3. Error bars are $\pm$ s.e.m.

Figure 1 blots

Figure 1b

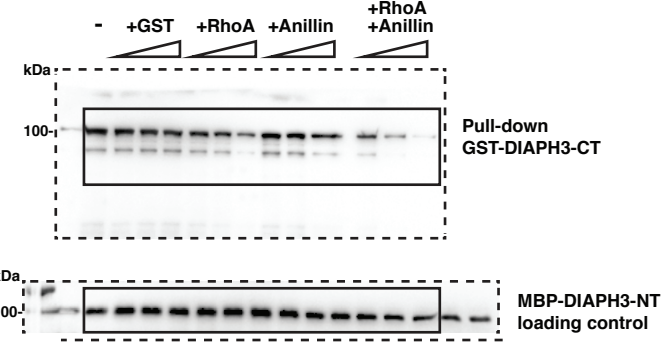


Figure 1c

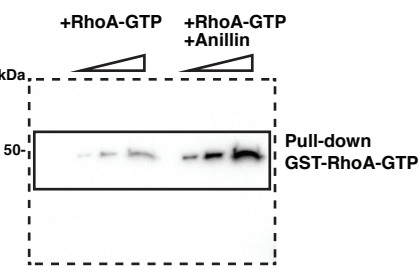


Figure 1d

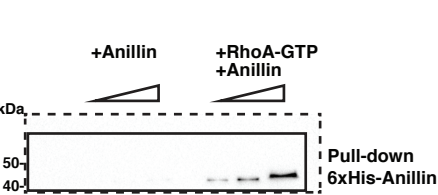


Figure 2 blots

Figure 3a

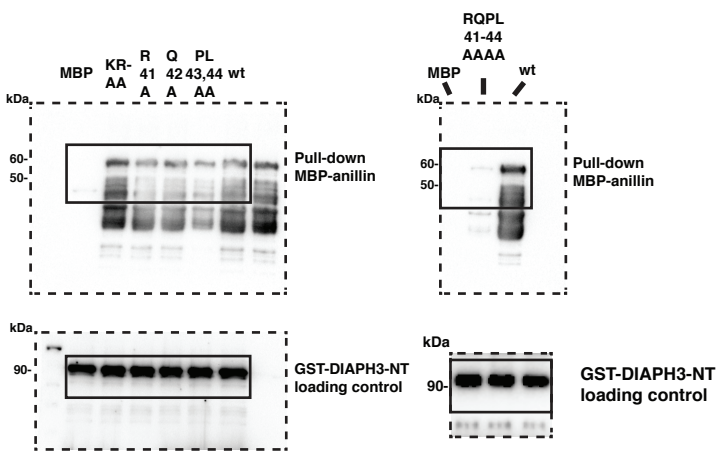


Figure 3c

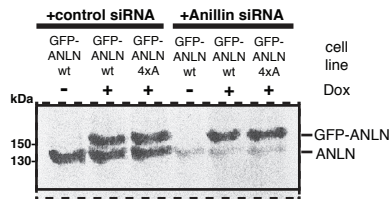


Figure 3d

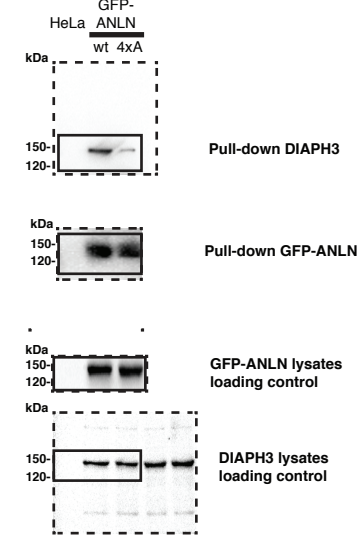


Figure 5 blots

Figure 5a

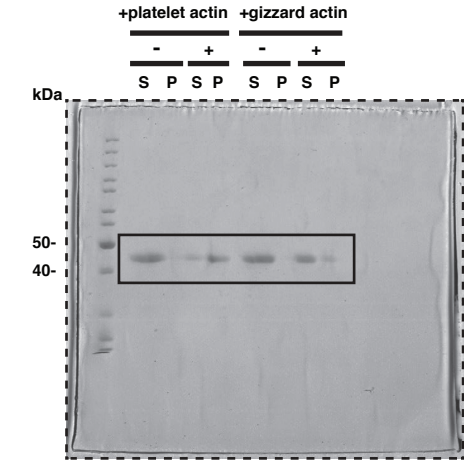
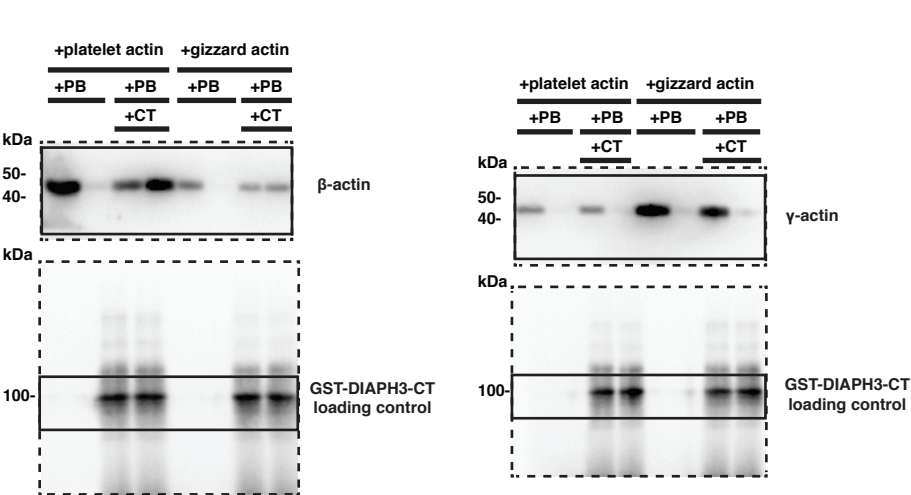
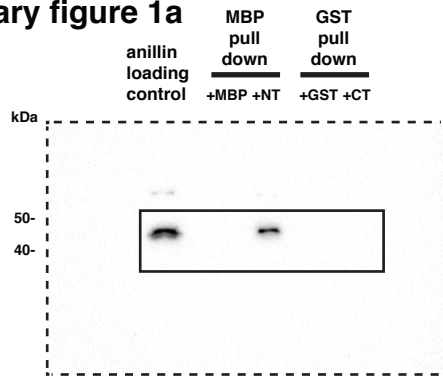


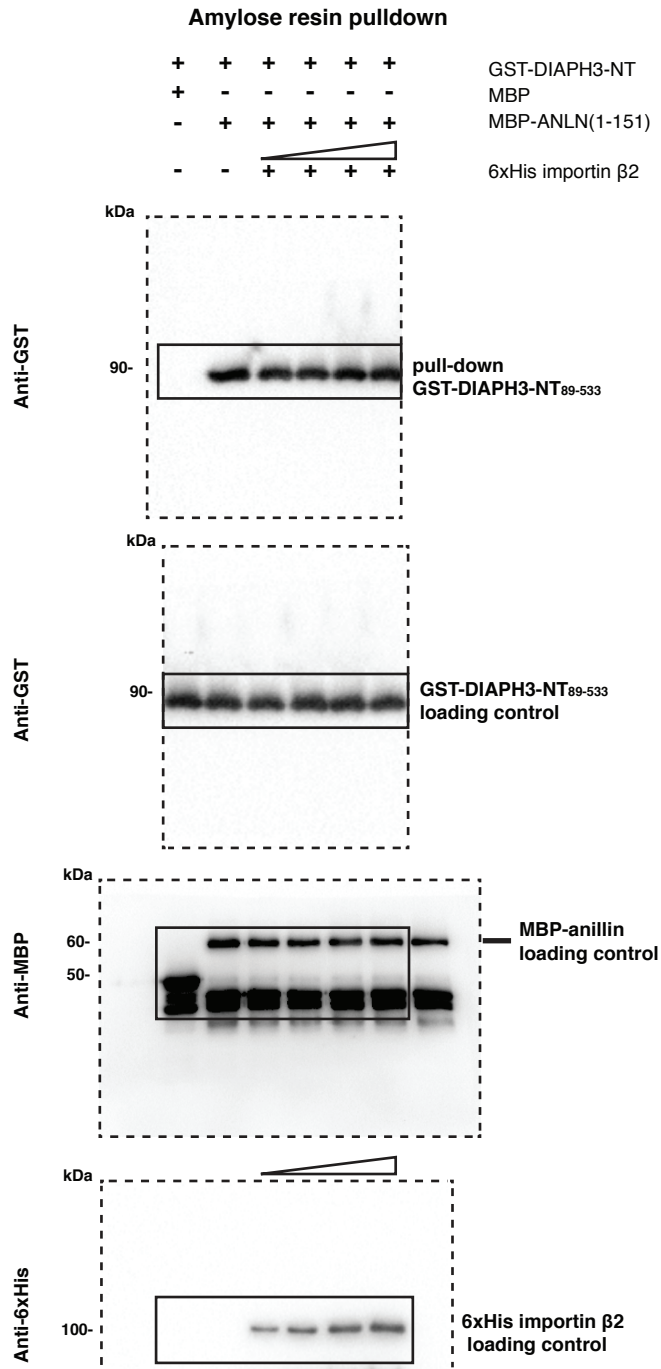
Figure 5b



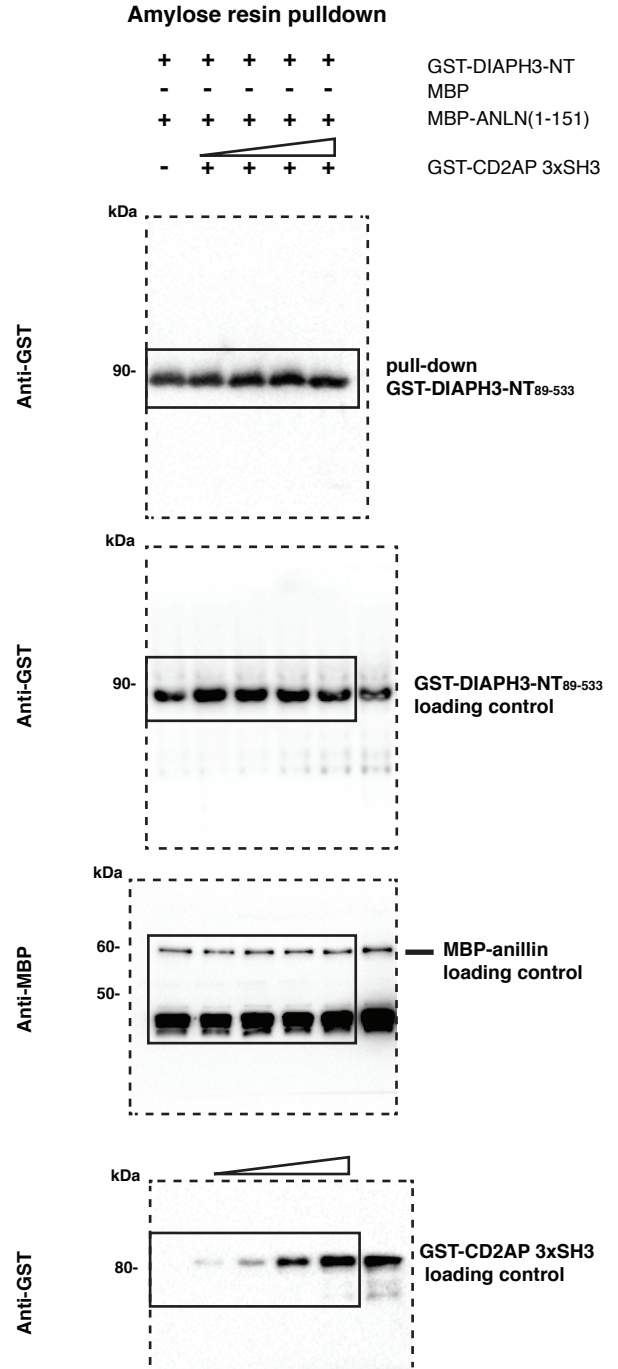
Supplementary figure 1a



Supplementary figure 1c



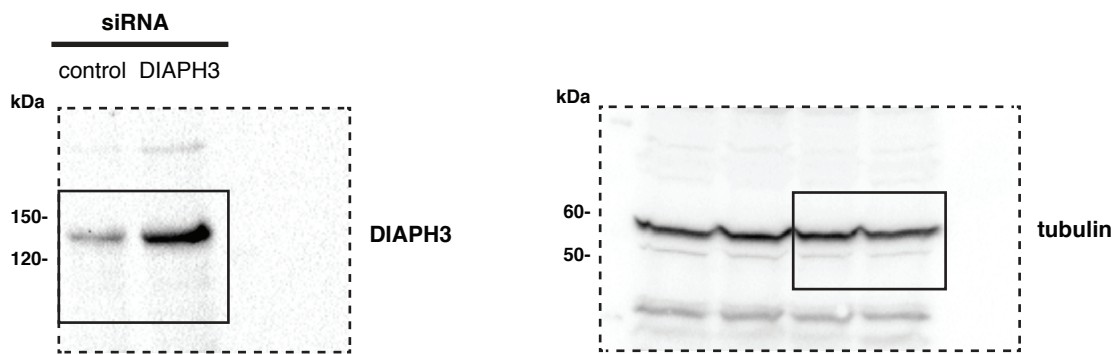
Supplementary figure 1d



Supplementary Figure 9. Uncropped images of blots in Supplementary Figure 1

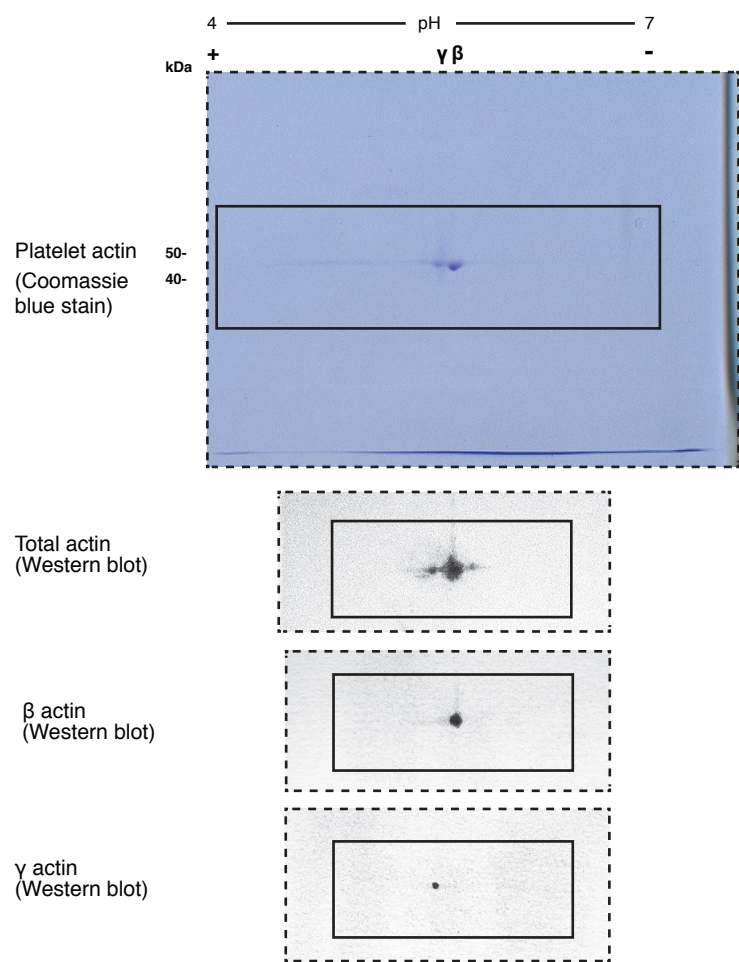
Supplementary Figure 3 blots

Supplementary figure 3a

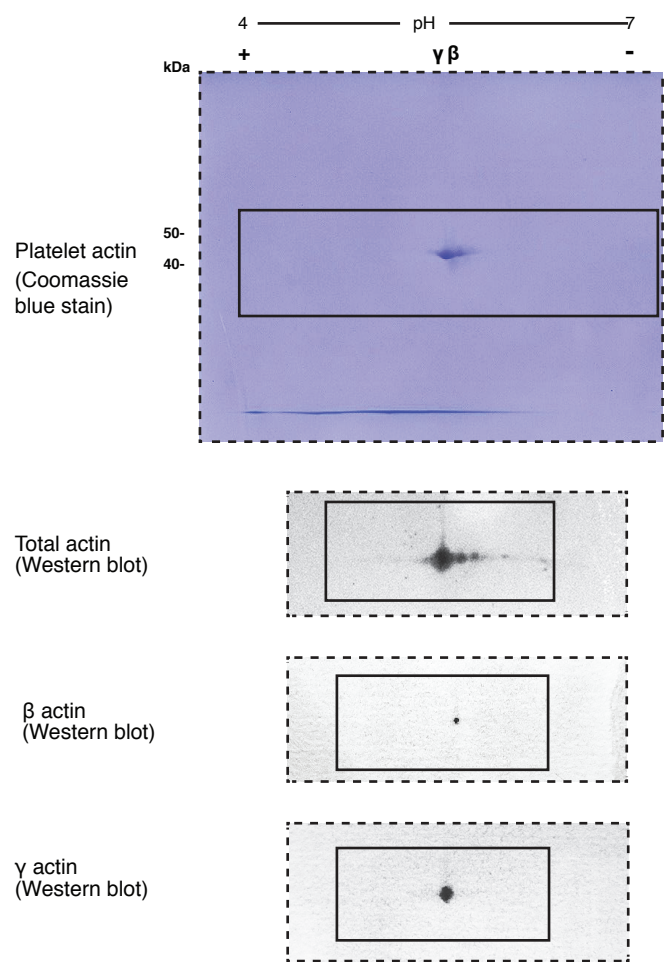


Supplementary Figure 4 blots

Supplementary figure 4b

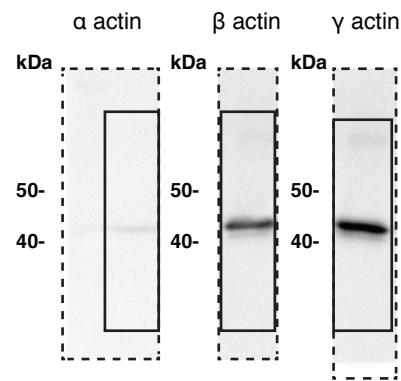


Supplementary figure 4c

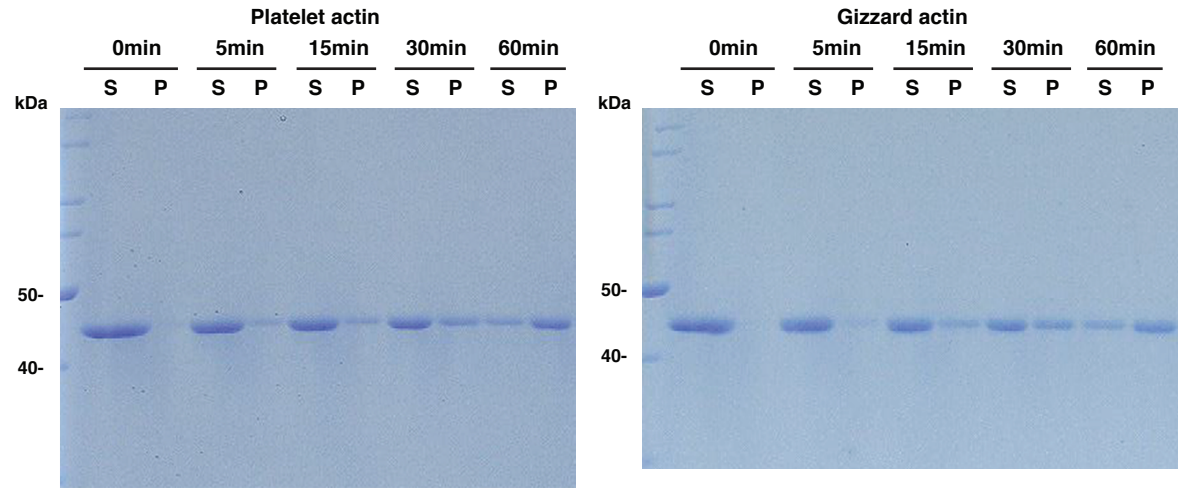


Supplementary Figure 10 Uncropped blots and gels from Supplementary Figures 3 and 4

Supplementary Figure 6c



Supplementary Figure 7 gels



Supplementary Figure 11. Uncropped blots and gels from Supplementary Figs 6 and 7

Supplementary Table 1: Applied DNA oligonucleotides

Name	Sequence (5' to 3')
5'_anillin	ATGGATCCGTTTACGGAGA
3'_anillin	TTAAGGCTTCCCAACAGGCTT
5'_anillin_Lic	GCTGGCGCTGGTGC GG GTGCCGGAATGGATCCGTTTACGGA
3'_anillin_Lic	TCCGTGGCGGCCTCGTCGTCGGGATTAAGGCTTCCCAACAGGC
fwd_anillin_22	AAAATGGCTGAGAGGCCCA
fwd_anillin_57	GAGAAATCTTGTACAAAACC
rev_anillin_57	TTCACCACCAGAGAGGGG
rev_anillin_91	TGTCGACTCAACTGGTTGTTT
rev_anillin_151	CTCTGCAAGTTTTTGCATAC
rev_anillin_300	AGTAGATTTCACTGGAGAAG
5'_mDia2	ATGGAACGGCACCAGCCG
3'_mDia2	TTATAAAGCTCGTAATCTTGCC
fwd_mDia2_89	CCACTTCCCAACCTGAAG
fwd_mDia2_561	CCTTTGCCTCCCTCTAAAGA
rev_mDia2_533	TTTTTTCTGCAATTCAGCCTGAGT
fwd_anillin_KR (38-39)-AA	CCAAGGTCTATGACTCATGCTGCAGCAGCTAGACAGCCA
rev_anillin_KR (38-39)-AA	TTCTGAAAGTGGCTGTCTAGCTGCTGCAGCATGAGTCAT
fwd_anillin_R41A	ATGACTCATGCTAAGCGAGCTGCACAGCCACTTTCAGAA
rev_anillin_R41A	TGCTTCTGAAAGTGGCTGTGCAGCTCGCTTAGCATGAGT
fwd_anillin_Q42A	ACTCATGCTAAGCGAGCTAGAGCACCCTTTCAGAAGCAA
rev_anillin_Q42A	GTTACTTGCTTCTGAAAGTGGTGTCTAGCTCGCTTAGCATG
fwd_anillin_PL (43-44)-AA	CATGCTAAGCGAGCTAGACAGGCAGCATCAGAAGCAAGTAAC
rev_anillin_PL (43-44)-AA	CTGCTGGTTACTTGCTTCTGATGCTGCCTGTCTAGCTCGCT
fwd_anillin_RQPL (41-44)-AAAA	TCTATGACTCATGCTAAGCGAGCTGCAGCAGCAGCATCAGAAGCAAGTAAC
rev_anillin_RQPL (41-44)-AAAA	GGGCTGCTGGTTACTTGCTTCTGATGCTGCTGCTGCAGCTCGCTTAGCATGAGT
fwd_Tom20-GFP-CT	ATGGACGAGCTGTACAAGAACTAGAAGAGTTTGAAGAG
rev_Tom20-GFP-CT	TCTTGAGTCGCGGCCTTATAAAGCTCGTAATCTTGC

fwd: forward primer **rev:** reverse primer **Lic:** Ligation independent cloning

CT: C-terminus of mDia2

Supplementary Table 2: Applied siRNA oligonucleotides

Name	Sequence (5' to 3')
3'UTR anillin	AGCUUACAGACUUAGCAU
NC	CGUUAUUCGCGUAUAAUACGCGUT
3' UTR DIAPH3	CCUUAUUGGGAUCUAUUAUACUACC

UTR: Untranslated region **NC:** Negative control